COMPARATIVE ANATOMY OF THE LEAF-BEARING CACTACEAE, XIII

THE OCCURRENCE OF WATER-SOLUBLE ANISOTROPIC BODIES IN AIR-DRIED AND ALCOHOL-DEHYDRATED LEAVES OF PERESKIA AND PERESKIOPSIS

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IN A PRELIMINARY SURVEY of the occurrence of crystals in the leaf-bearing Cactaceae (Bailey 1961), I noted the common presence in various species of *Pereskia* of brown isotropic bodies during earlier stages of the clearing of leaves in three per cent sodium hydroxide. Although isotropic in polarized light, the radial striations and occasional concentricities of these bodies superficially resembled those that occur in certain "sphero-crystals" of calcium oxalate reported upon in the Cactaceae by Mobius (1885). They differed fundamentally, however, from the abundantly occurring druses and other crystalline forms of calcium oxalate in the family which are insoluble and retain their anisotropy in dilute solutions of sodium hydroxide. I concluded that the chemical composition and the factors involved in the formation of the isotropic bodies merited detailed investigation.

Having obtained more extensive collections of *Pereskia*, I now find that of 14 putative species of the genus all exhibit a tendency to form more or less numerous bodies comparable to those previously reported upon (Bailey 1961). This is true in the case of leaves from herbarium specimens of 11 species collected from plants growing in their native habitats and, likewise, in the case of leaves of 14 species preserved in formalin acetic alcohol (FAA).[2] Furthermore, the normal living leaves of eight species, although they have formed abundant druses of calcium oxalate, do not contain such bodies, but tend to develop them when the leaves are air dried or are transferred to alcohol varying in dilutions of from 95 to 50 per cent.

In the ordinary white light of a microscope the crystalline contents of the bodies are colorless. In polarized light they are as strikingly birefringent as the druses of calcium oxalate (FIG. 1). When dehydrated leaves are placed in a dilute solution of sodium hydroxide, the bodies rapidly lose their birefringence and commonly turn brown (FIG. 2). Upon prolonged treatment in alkali, the isotropic residues of the aniso-

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[2] Forty per cent formaldehyde, five parts: glacial acetic acid, five parts: 50 per cent ethyl alcohol, ninety parts.

tropic bodies ultimately lose their striated and other internal structural features and dissolve (Bailey 1961, *figs. 30–33*). This behavior is in marked contrast to that of druses and small individual units of calcium oxalate which, in the same leaves, remain insoluble and retain their anisotropy (FIG. 2). That the birefringent bodies are not composed of calcium oxalate is indicated, not only by their behavior when transferred to dilute sodium hydroxide, but also by their *solubility in water at ordinary room temperatures*.

In *Pereskia*, the size, form, and abundance of the water-soluble birefringent bodies varies more or less extensively in different collections of a taxon, in different leaves of the same plant, and in different layers of the same leaf. The larger more conspicuous bodies commonly are composed of a group of adjacent cells filled with compact birefringent contents which frequently exhibit fine striations oriented radially toward the center of the body (FIG. 5). Smaller bodies of spherical or angular form have a similar compact crystalline composition, but fewer adjacent cells are concerned in their development. In some cases, single large independently occurring cells of the mesophyll are not completely filled with crystalline contents, but may contain one or more small, spherical, radially striated bodies or hemispherical striated ones attached to the walls of the cell.

When many diversely dehydrated leaves of *Pereskia* are examined, some of them contain, in addition to larger bodies, minute water-soluble, birefringent units diffused throughout parts of the mesophyll (FIG. 4). These units vary markedly in crystalline form from rotund to rectangular, diamond-shaped, slender boat-shaped, and thin angular platelets. Some of the larger ones appear to arise by fusion of smaller units forming rings or spheres with or without isotropic interiors. These birefringent units, not only are water soluble, but also lose their anisotropy and turn brown in dilute sodium hydroxide.

At times, certain crystalline forms of the smaller units tend to be aggregated in varying degrees of compactness. For example, as illustrated in FIG. 3, single diamond-shaped crystals may be surrounded by a layer of aggregated crystals of different form. In other cases, groups of adjacent cells contain aggregations of small birefringent units in varying degrees of compactness. There appear to be transitional forms of crystallization between such structures and the commonly occurring extreme form illustrated in FIG. 5. At times, large bodies of the latter general form, having compact radially striated crystalline interiors, are jacketed by flat, more or less rectangular, birefringent units which are loosely aggregated and haphazardly oriented.

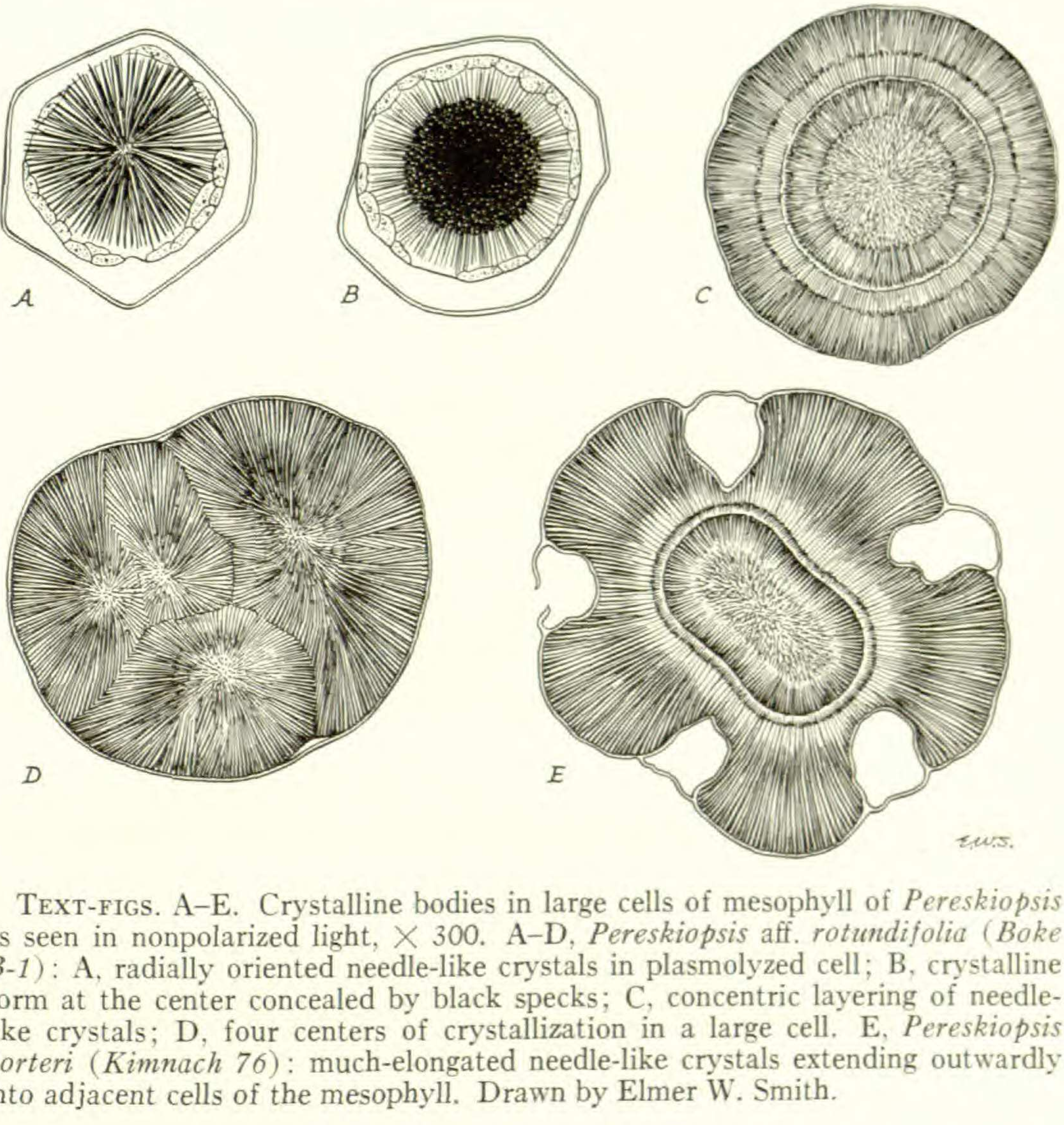
I have not encountered slender, much elongated, needle-like crystals occurring independently in *Pereskia*. However, particularly when living leaves are dehydrated in 95% alcohol, aggregations of such crystals may be formed in a few adjacent cells subtending stomata (FIG. 6). Where the elongated crystals are oriented parallel to one another, the aggregation may superficially resemble raphides of calcium oxalate, but the fascicles are not confined to single enlarged cells (i.e., idioblasts), are not embedded in mucilage, and are water soluble. Very infrequently one encounters a radially oriented aggregation of such needle-like crystals in a single unusually large cell of the mesophyll (FIG. 7).

It is evident from observations recorded in preceding paragraphs that the birefringent structures induced during dehydration of the leaves of *Pereskia* exhibit more extensive variability in crystalline form than do druses of calcium oxalate persisting from the living condition in the same leaves. However, it is significant that, in spite of the diversity in the induced forms of crystallization, all of them are characterized by their solubility in water and by their similar behavior when transferred to dilute sodium hydroxide. It is not obvious at present why some dehydrated collections of a taxon or clone form the bodies, whereas others do not; why some leaves of a shoot contain them, when adjacent leaves are without them; and why some parts of a leaf have them, when other parts are devoid of them. Nor is it evident what factors are concerned in producing the variability in forms of crystallization. More detailed and extensive investigations, particularly adequate chemical analyses, starting with normal living leaves are highly desirable for elucidating such uncertainties.

In 36 collections of *Pereskiopsis* of varying taxonomic affinities, kindly collected and preserved for me in FAA by Boke, Moran, Kimnach, and others, a majority of 20 has leaves and occasionally young stems containing water-soluble birefringent bodies of varying abundance, sizes, and diversified crystalline forms. The largest of these bodies, up to and exceeding 600 microns in diameter, are much larger than the largest of those that occur in *Pereskia* (compare the contrasting sizes at the same magnification in FIGS. 1, 8, & 9). In addition, the larger birefringent bodies of *Pereskiopsis* differ markedly in their internal crystalline composition from those of *Pereskia*.

In *Pereskiopsis*, the larger bodies of multicellular origin tend to be rings or disks of varying thickness oriented periclinally in relation to the external surfaces of leaves and young stems. They are readily detectable without magnification, appearing superficially as embossed white pustules in the surfaces of leaves and stems which shrink during dehydration in FAA. In some of these bodies, all of the cells in the interior of the body are devoid of crystalline content, except for cells which contained druses of calcium oxalate in the living leaf or stem. In such a body, the isotropic core is jacketed by an anisotropic layer of varying thickness which appears as a birefringent ring in polarized light. More frequently the bodies have a cell with water-soluble birefringent contents at its center surrounded by an isotropic zone (which may contain water-insoluble druses of calcium oxalate) and, in turn, by an outer birefringent one having more or less numerous outwardly projecting bulges (FIG. 9). Less frequently most cells in the interior of a body may contain loosely aggregated water-soluble crystals (FIG. 8).

The individual crystals in these large birefringent bodies vary markedly in size, form, degree of aggregation, and orientation. Particularly in the outer birefringent zone of the bodies, many of the constituent cells exhibit a conspicuous tendency to contain slender, elongated, more or less needle-like crystals oriented radially toward the center of the body. Similar forms of crystallization and orientation may be present in the central cell of a body. In the case of the large outwardly bulging cells illustrated in FIG. 9, some of them may have needle-like, radially oriented crystals in their inner or outer parts, whereas remaining parts of the cell contain minute widely diffused birefringent specks resembling "crystal sand."



TEXT-FIGS. A–E. Crystalline bodies in large cells of mesophyll of *Pereskiopsis* as seen in nonpolarized light, × 300. A–D, *Pereskiopsis* aff. *rotundifolia* (*Boke B-1*): A, radially oriented needle-like crystals in plasmolyzed cell; B, crystalline form at the center concealed by black specks; C, concentric layering of needle-like crystals; D, four centers of crystallization in a large cell. E, *Pereskiopsis porteri* (*Kimnach 76*): much-elongated needle-like crystals extending outwardly into adjacent cells of the mesophyll. Drawn by Elmer W. Smith.

Some dehydrated collections of *Pereskiopsis* contain smaller, more nearly spherical bodies. Some of these, when viewed in optical section (FIG. 10), resemble the larger body illustrated in FIG. 9. A single cell with birefringent contents at the center of the body is surrounded by an isotropic layer and, in turn, by an outer anisotropic one. In other cases, the bodies are birefringent throughout, their central part exhibiting very narrow anisotropic concentricities partly concealed in FIG. 11 by four positions of extinction when viewed in polarized light. The crystalline contents of the cell in the center of such bodies may be deposited at times in similar concentricities (FIG. 10), whereas in other cases the central cell contains larger elongated units resembling those that occur in cells of the external parts of the bodies (FIG. 11). It is significant in this connection, however, that the longer axis of the crystalline units — regardless of extreme variations in size and form — tend to be radially oriented toward the center of the bodies. It should be noted, in addition, that cells, particularly in the interior of the larger bodies, not infrequently contain black granular contents which tend to obscure internal crystalline configuration when the bodies are viewed in polarized light.

Some of Dr. Boke's collections of *Pereskiopsis* preserved in FAA in their native habitats (particularly in his *B-1*, *B-18*, *B-20*, *B-22*, and *B-31*, with putative affinities to *P. aquosa*, *P. chapistle*, *P. rotundifolia*, or *P. spathulata*) contain, in addition to multicellular bodies, large independently distributed cells with water-soluble crystalline contents. Some of these cells (TEXT-FIG. A) have slender, elongated, radially oriented units resembling the configuration that occurs so infrequently in *Pereskia* (FIG. 7). Occasionally a large cell may contain several bodies of similar crystalline form (TEXT-FIG. D). The contents of other cells of the same leaf (TEXT-FIG. C) commonly exhibit conspicuous concentricities, due apparently to variations in the size or form of the crystalline units in successive zones of their deposition. In some cases the longer needle-like crystals of the external zone extend outwardly into parts of adjacent cells forming expanded bodies of more or less conspicuously lobed or angular forms (TEXT-FIG. E). At times, the inner parts of some of the cells contain numerous black isotropic specks (TEXT-FIG. B) which tend to obscure the anisotropy in the interior of concentric configurations.

DISCUSSION AND CONCLUSIONS

The water soluble birefringent bodies of *Pereskia* and *Pereskiopsis* are formed during dehydration of the "sap" of living cells. In *Pereskia*, the larger bodies are remarkably similar regardless of whether leaves are air dried, as in the case of herbarium specimens, immersed in 95%, 70%, and 50% ethyl alcohol, or preserved in FAA. In none of my numerous collections of different species of the genus do larger crystalline bodies comparable to those of *Pereskiopsis* occur. Although highly variable, the forms of crystallization differ so consistently in *Pereskia* and *Pereskiopsis* as to provide, even in herbarium specimens, an additional criterion for separating the genera taxonomically.

Young green stems in some of my collections of *Opuntia* (preserved in FAA) contain water soluble birefringent bodies of sizes and crystalline forms resembling those that occur in *Pereskiopsis*, suggestive of possible taxonomic relationship. Furthermore, the "spherocrystals" in *Echinocactus*, *Mamillaria*, and *Anhalonium* observed and briefly discussed by Lauterbach (1889) and Michaëlis (1896) were found in tissues treated with alcohol. It now appears that these anisotropic bodies, unlike normally occurring druses and other forms of calcium oxalate, belong in a special water soluble category. The occurrence of such bodies in all three subfamilies or tribes of the Cactaceae at least suggests that a tendency for their formation during dehydration of the "sap" of living cells may be widely distributed throughout this characteristically xerophytic family.

Since the publication of Schleiden's (1845) classical paper, the Cactaceae have proved to be a family having extraordinarily abundant druses and other water insoluble crystalline forms of calcium oxalate in their tissues, indicative, in turn, of the production of excessively large amounts of oxalic acid in their metabolism. It is significant in this connection that in living cacti, crystals of calcium oxalate occur in the mesophyll of leaves (where present) and in parenchymatous cells of the pith, cortex, xylem, and phloem of both stems and roots. In contrast to this, the water soluble crystals formed during dehydration of "sap" in *Pereskia* and *Pereskiopsis* occur primarily in the chlorenchyma of leaves and in the cortex of green stems which function in photosynthesis.

Unfortunately, owing to numerous complexities and uncertainties, it does not appear possible at present to determine the exact chemical composition of the water soluble bodies merely by their forms of crystallization. For example, upon the basis of superficial resemblances in forms of crystallization, it has been suggested by Lauterbach (1889) and others that the birefringent bodies in dehydrated tissue of the Cactaceae are composed of inulin or related substances. Such is not the case in collections available to me where tests for such composition of the crystalline bodies are negative. That calcium is involved in the chemical composition of the larger birefringent bodies of *Pereskiopsis* may be demonstrated by treatment in sulphuric acid. When transferred to this acid the birefringence of the bodies is rapidly reduced, but is quickly restored by the formation of crystals of calcium sulphate. The transition to calcium sulphate tends to occur more rapidly than in druses of calcium oxalate present in the same tissue.

It is of interest in these connections that similar forms of crystallization in alcohol have been encountered in certain representatives of other families of angiosperms, viz. cactus-like euphorbias, *Mesembryanthemum* (Aizoaceae), *Basella* (Basellaceae), *Ceropegia* and *Stapelia* (Asclepiadaceae), *Galtonia* (Liliaceae), and *Nolana* (Nolanaceae). Belzung (1893), Belzung and Poirault (1892) and Mirande (1898), following the earlier investigations of Hansen (1888), Leitgeb (1888) and others, concluded upon the basis of extensive microchemical and other tests that calcium malate and calcium "malophosphate" (?) are concerned in such plants during the formation of water soluble crystals when their tissues are dehydrated in alcohol. A similar conclusion was reached subsequently by Kean (1931) in his investigation of *Mesembryanthemum*.

Although these investigations are suggestive rather than conclusive from modern chemical points of view, they do indicate that the water soluble crystals are in all probability calcium salts of organic acids, occurring in close association with calcium phosphate. It is unlikely that the crystals are composed solely of calcium phosphate, as suggested by earlier investigators, since such crystals are almost insoluble in water as are the crystals of calcium oxalate. That dehydration in alcohol is not essential for their formation is clearly indicated by their typical occurrence in the dehydrated cellular "sap" of air dried leaves, for example in herbarium specimens.

At present, cumulative circumstantial evidence from these phylogenetically highly specialized genera, in correlation with the behavior in the characteristically xerophytic Cactaceae, raises a fundamentally significant question. Are there evolutionary changes in the metabolism of angiosperms (viz. those having excessive succulence) which lead to the formation of unusually large amounts of such organic acids as oxalic, malic, etc.? The phenomena are highly complex and variable and involve various uncertainties. An adequate answer to the question will involve active and sustained cooperation between botanists and chemists.

Preliminary observations on the only normal living leaves of *Pereskiopsis* available to me thus far (from Dr. Boke's culture of his *B-18*) suggest that this genus may provide more favorable material for studying successive stages in the formation of birefringent bodies during dehydration than does *Pereskia*. A more detailed discussion of the phenomena will be undertaken when living leaves and young stems of other species of *Pereskiopsis* become available.

LITERATURE CITED

BAILEY, I. W. 1961. Comparative anatomy of the leaf-bearing Cactaceae, III. Form and distribution of crystals in *Pereskia, Pereskiopsis* and *Quiabentia*. Jour. Arnold Arb. **42**: 334–346.

BELZUNG, E. 1893. Nature des sphérocristaux des Euphorbes cactiformes. Jour. Bot. Morot **7**: 221–229, 261–267.

——— & G. POIRAULT. 1892. Sur les sels de l'*Angiopteris evecta* et en particulier le malate neutre de calcium. Jour. Bot. Morot **6**: 286–298.

HANSEN, A. 1888. Über Sphärokrystalle. Arbeit. Bot. Inst. Würzburg **3**: 92–122.

KEAN, C. I. 1931. Artificial crystals in preserved tissues of *Mesembryanthemum*. Trans. Bot. Soc. Edinb. **30**: 43–45.

LAUTERBACH, H. 1889. Untersuchungen über Bau und Entwicklung der Sekretbehälter bei den Cacteen. Bot. Centralbl. **37**: 257–264, 289–297, 329–336, 369–375, 409–413.

LEITGEB, H. 1888. Über sphärite. Mitt. Bot. Inst. Graz **1**: 257–260.

MICHAËLIS, P. 1896. Beiträge zur vergleichenden Anatomie der Gattungen *Echinocactus, Mamillaria* und *Anhalonium*. Doctoral Thesis. Halle.

MIRANDE, M. 1898. Contribution à l'étude du malate de neutre calcium et du malophosphate de calcium dans les végétaux. Jour. Bot. Morot **12**: 6–12, 32–42, 58–60.

MÖBIUS, M. 1885. Sphaerokrystalle von Kalkoxalate bei Cacteen. Ber. Deutsch. Bot. Ges. **3**: 178–182.

SCHLEIDEN, M. J. 1845. Beiträge zur Anatomie der Cacteen. Mém. Acad. Imp. Sci. St.-Pétersbourg **4**: 335–380.

EXPLANATION OF PLATES

PLATE I

FIGS. 1–4. Parts of leaves of *Pereskia* photographed in polarized light. 1, *Pereskia aculeata*, showing birefringence of large water-soluble bodies and anisotropy of small druses of calcium oxalate in dehydrated leaf, × 114; 2, *P. aculeata*, showing loss of birefringence by the water-soluble bodies and retention of anisotropy by druses of calcium oxalate after treatment in dilute sodium hydroxide, × 114 (in this leaf both the water-soluble bodies and the druses were larger than in FIG. 1); 3, *Pereskia corrugata*, diamond-shaped crystals surrounded by aggregations of small crystals of different form, × 180; 4, *P. corrugata*, showing diffusely distributed minute water-soluble crystals, × 114.

PLATE II

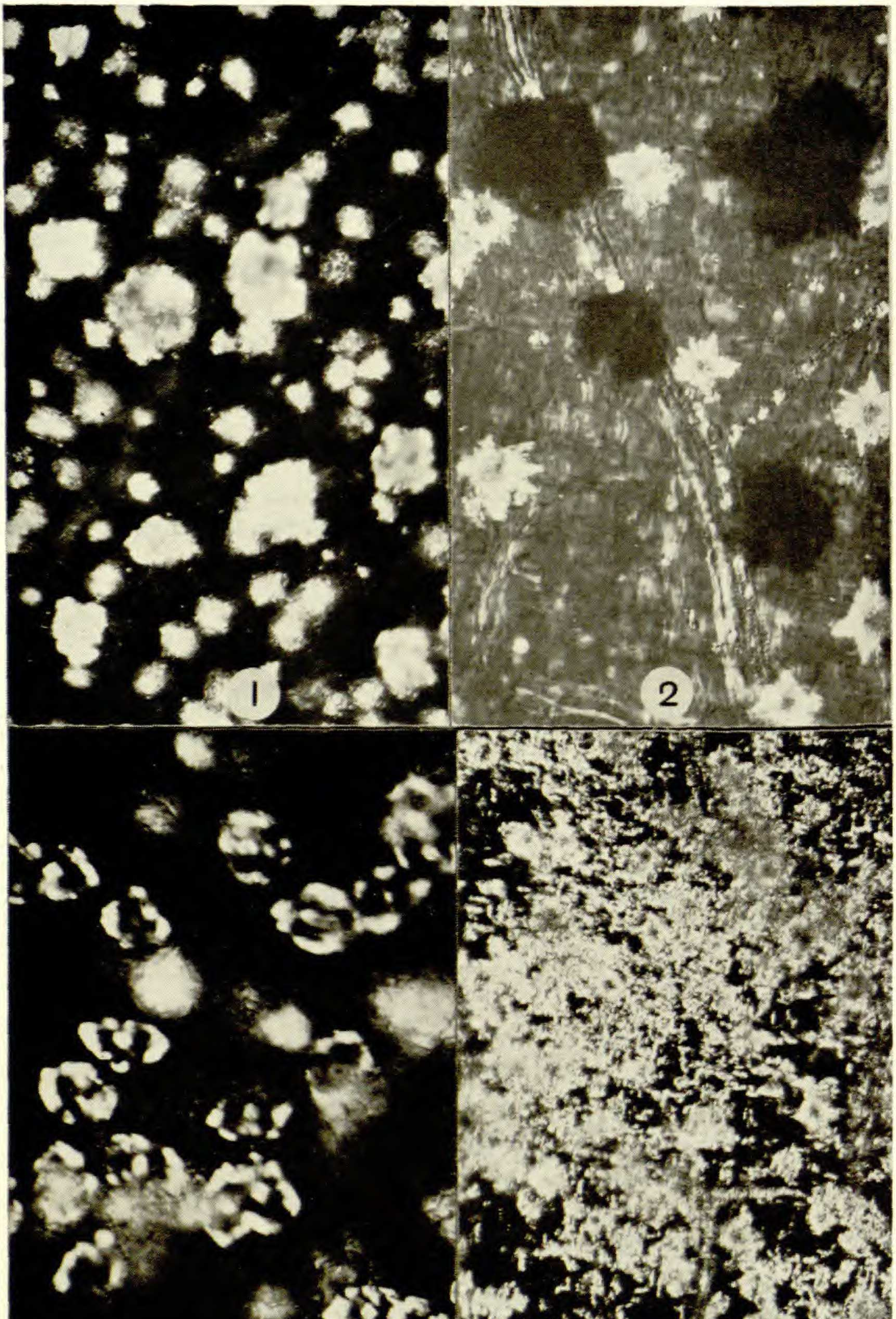
FIGS. 5–7. Water-soluble birefringent bodies of *Pereskia* photographed in non-polarized light, × 260. 5, *P. corrugata* showing large compact radially striated body; 6, *P. sacharosa* showing fascicles of needle-like crystals in cells subtending stomata; 7, *P. pititache* showing radially oriented needle-like crystals in a single large cell.

PLATE III

FIGS. 8 & 9. Large water-soluble birefringent bodies of *Pereskiopsis* (*Boke B-22*) preserved in FAA, photographed in polarized light, × 114; 8, crystals diffused throughout the body; 9, body having a cell at the center with birefringent contents, surrounded by an isotropic layer (except for druses of calcium oxalate persisting from the living leaf) and jacketed externally by a birefringent zone.

PLATE IV

FIGS. 10 & 11. Smaller more nearly spherical water-soluble birefringent bodies of *Pereskiopsis* photographed in optical section in polarized light. 10, From Boke's collection *B-20* preserved in FAA, × 330; 11, from Boke's collection *B-1* preserved in FAA, × 430.



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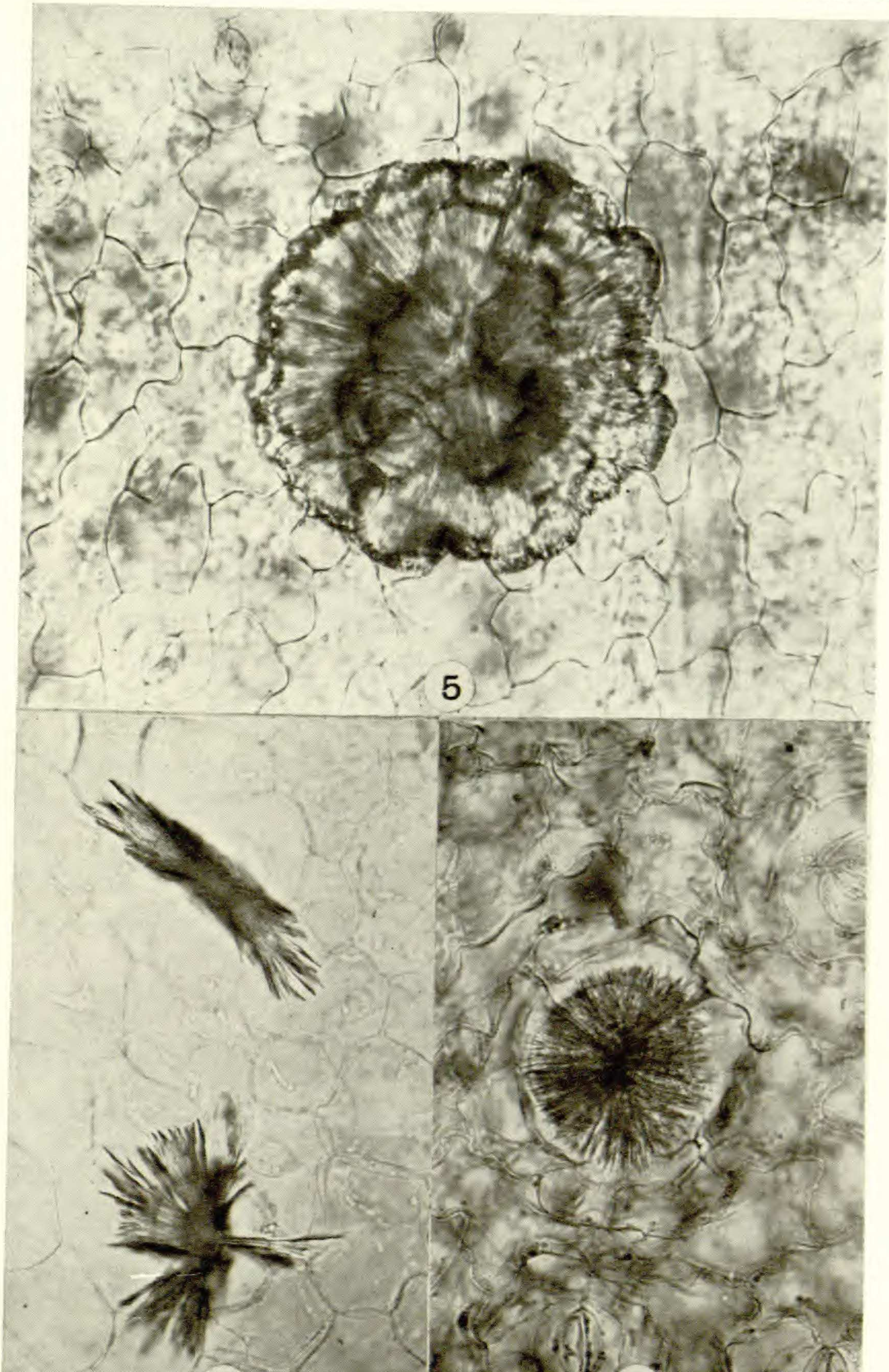

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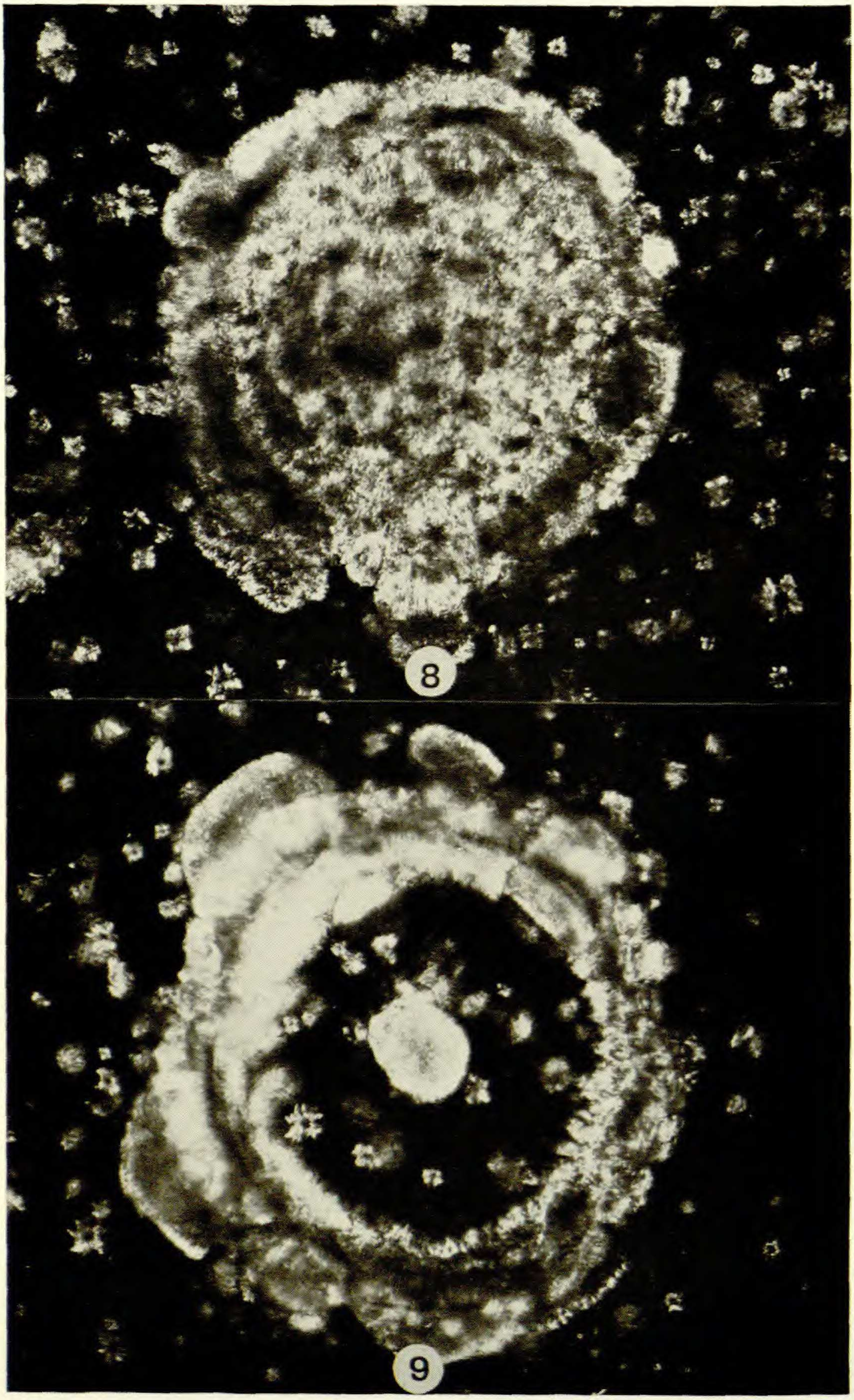

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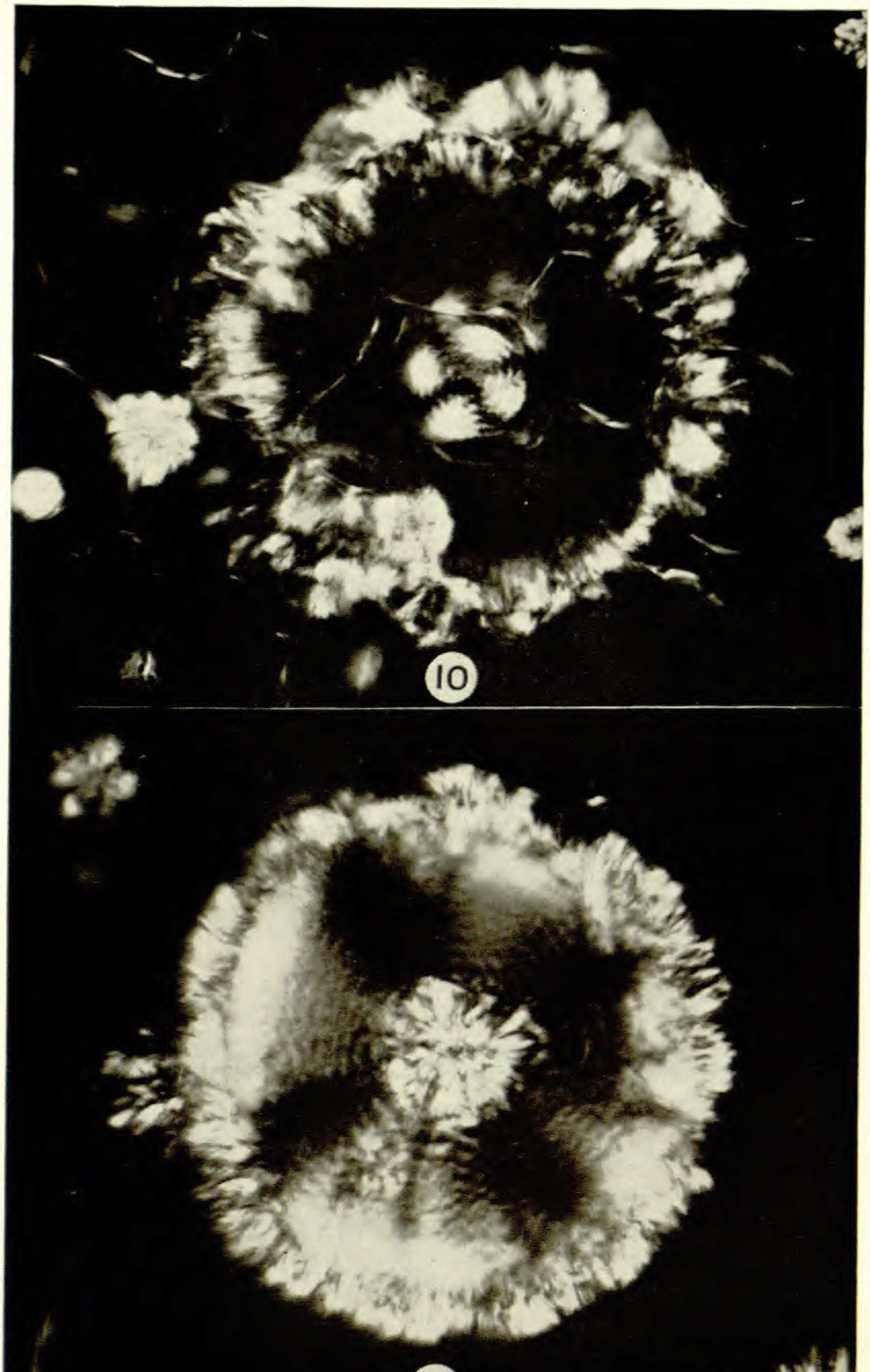

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